Applied Soil Ecology 124 (2018) 155–162

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effect of *Thermomyces* fungal endophyte isolated from extreme hot desert-adapted plant on heat stress tolerance of cucumber

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ARTICLE INFO

Keywords:
Plant-endophytes association
Heat stress
Photosynthesis
Antioxidant

ABSTRACT

With the increasing effects of global climate change and population growth, there is a high need for new agricultural practices to maximize efficiencies and reduce the efforts needed for developing new crop varieties that can withstand various abiotic stress. Plant-microbe association to optimize plant growth and increase plant host stress tolerance may play an essential role in improving environmental sustainability and economic viability, especially in the arid land habitats. In the present study, photosynthetic responsiveness was selected to demonstrate that a theromophilic endophytic (CpE) fungus isolated from the hot desert-adapted delile (*Cullen plicata*) roots can mediate heat stress tolerance in cucumber plants grown in the field during the summer season in Egypt. Initially, the CpE isolate was identified using an internal transcribed spacer, revealing 92% sequence homology with *Thermomyces* sp. Then the cucumber seeds were treated with CpE endophyte spore and allowed to grow in a growth chamber for three weeks before transplanting into the field station from May to July. The CpE treatment eliminates the adverse effects of heat stress on cucumber plants by maintaining the maximum quantum efficiency of photosystem II, photosynthesis rate, water use efficiency and increase root length relative to untreated plants. In addition, CpE treatments induced the significant accumulation of total sugars, flavonoids, saponins, soluble proteins and antioxidant enzyme activities in comparison with untreated cucumber plants. Our finding provides novel insights into the eco-physiological mechanisms of thermophilic CpE-mediated heat stress tolerance in cucumber, which signify the prospective applications of arid land-adapted endophytes in agricultural systems.

1. Introduction

Impacts from recent climate-related conditions, such as heat-waves and persistent drought in addition to the reductions in the quantity and quality of arable land and water will change the current and future world agricultural production (Abdelrahman et al., 2017a, 2017b). Heat waves are predicted to become more frequent and persistent more prolonged than what is being currently observed in recent years, especially during the summer season, affecting ecosystem productivity and diversity (Hatfield and Prueger, 2015). Despite these encounters, global food production will have to rise by 70% to meet the mandate of an anticipated increase in population growth to 9 billion by 2050 (Stratonovitch and Semenov, 2015; Abdelrahman et al., 2017b), which will push agriculture sustainability to the edges in the coming years. To meet these challenges, new discoveries and research technologies aim to maximize efficiencies and reduce the inputs needed for developing new crop varieties and maintain water usage will be essential to improve agriculture sustainability and food security. Traditional breeding programs to develop abiotic stress-resilient crops remain insufficient due to the lack of abiotic stress resistance germplasms and time-consuming (Zhang, 2016). Recently, the association between the plant and the extreme habitat-adapted endophytes or beneficial microorganisms is becoming a well-established strategy to enhance plant survival capacity and increase yield under various abiotic and biotic stresses (Khan et al., 2012; Jogaiah et al., 2013; Abdelrahman et al., 2016; Gill et al., 2016; Khan et al., 2016). The genetic base of abiotic stress resistance in extremophilic fungi, particularly thermophilic one is a unique genetic resource, enabling the plant to survive under abiotic stress conditions (Zhang, 2016). For example, *Thermomyces* sp. is a thermophilic fungus that can survive at 62 °C due to the presence of ubiquitin degradation, histone acetylation/deacetylation, and poly ADP-ribosylation pathways, which play a crucial role in *Thermomyces* response to heat stress (Mchunu et al., 2013). However, the beneficial role of this thermophilic fungi to induce heat stress tolerance in crop

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https://doi.org/10.1016/j.apsoil.2017.11.004
Received 27 September 2017; Received in revised form 1 November 2017; Accepted 3 November 2017
Available online 14 November 2017
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plants remain elusive.

Heat stress may cause considerable impacts on crop plants by disturbing essential physiological functions, including increase in membrane fluidity, protein denaturation, level of reactive oxygen species (ROS), inactivation of mitochondrial and chloroplast enzyme activities, as well as decline in photosystem II (PSII)-mediated electron transport (Gururani et al., 2015; Guo et al., 2016; Feller, 2016; Abdelrahman et al., 2017a). Photosynthetic process is the most thermosensitive structure of plant adaptation to heat stress, and heat stress considerably hampers the photosynthesis process in many plant species by changing the organelle structures and concentrations of several pigments and enzymes involved in this process as well as stomatal dynamic (Hatfield and Prueger, 2015; Ding et al., 2016; Wang et al., 2016). In addition, heat stress induces the accumulation of ROS, leading to the destruction of plant lipids, proteins, and carbohydrates (Li et al., 2014a,2014b; Abdelrahman et al., 2015; Harsh et al., 2016) thus, plants have evolved antioxidant defense systems which include non-enzymatic (flavonoids, ascorbic acid, and glutathione) and enzymatic antioxidants to scavenge the excess level of ROS and maintain cell ROS homeostasis (Abdelrahman et al., 2014; Harsh et al., 2016; Pandey et al., 2017).

Responses to high temperature varied among crop plants throughout their life cycle and could be species-specific (Hatfield and Prueger, 2015; Abdelrahman et al., 2017a). Cucumber (*Cucumis sativus* L.) is one of the important horticultural crops and highly sensitive to heat stress, especially at the vegetative stage (Ding et al., 2016; Li et al., 2016). Therefore, cucumber is strongly affected by heat stress and drought conditions when cultivated in the spring-summer season relative to the winter-spring, resulting in a shortened harvested time with the substantial decline in the quantity and quality (Li et al., 2014a,2014b). In this context, we investigated the physiological role of a thermophilic endophytic fungus (CpE) isolated from the roots of an extreme hot desert-adapted delile plant (*Cullen plicata*) to induce heat stress tolerance in cucumber plants grown under gradual filed temperature increase from 25 °C to 40 °C, during the summer season in Egypt. The response of photosynthetic process, including the maximum quantum efficiency of PSII photochemistry (F_v/F_m), photosynthesis rate (P_n), transpiration rate (T_r), leaf intercellular CO_2 concentration (C_i), stomatal conductance (G_s), water use efficiency (WUE) in CpE-treated and untreated cucumber plants at different temperatures were examined. Since the antioxidant potential of plant has received much attention as important adaption mechanism to abiotic stress, the ROS-scavenging enzyme activities, including phenylalanine ammonia lyase (PAL; EC 4.3.1.24), sodium dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and peroxidase (POX; EC 1.11.1.7) in addition to total saponin, flavonoid, carbohydrate and protein levels in CpE-treated and untreated cucumber plants were determined. Moreover, the phenological responses such as leaf fresh weight (FW), leaf dry weight (DW), leaf water content and root length were also evaluated under respective conditions. This study provides novel insights into the physiological mechanisms of thermophilic CpE-mediated heat stress tolerance in cucumber, which will facilitate the cultivation of heat-tolerant cucumber and accelerate the translation of primary physiological data into meaningful information for crop stress improvement.

2. Materials and methods

2.1. Isolation of endophyte CpE from the desert soil-adapted plant

Fresh delile plant materials were collected from extreme hot desert soil at Aswan University (39.59°N 32.82°E). Delile plant roots were placed in sealable bags and transported to the laboratory for processing within 12 h of collection. Roots were initially rinsed in tap water to eliminate surface soils and then surface-sterilized by successive immersion in 95% ethanol (10 s), 2% sodium hypochlorite (3 min) and 70% ethanol (2 min). Surface-sterilized roots were cut into approximately ~5 mm pieces under sterile conditions. Root pieces were allowed to surface-dry under sterile conditions prior to placement into growth media. Five pieces of root were placed on potato dextrose agar (PDA) plate and incubated at 45 °C for one week (Shivanna et al., 1996), and the observed single colony was isolated and coded as CpE.

2.2. Molecular characterization of the endophyte isolate and phylogenetic analysis

Total genomic DNA was extracted and isolated using SolGent purification bead (Solgent Co. Ltd., Daejeon, Korea). The polymerase chain reaction (PCR) was carried out to amplify the nuclear ribosomal internal transcribed spacers (ITS) using ITS1-F and ITS4-R primers: (ITS1-F: 5′-CCGTAGGTGAACCTGCGG-3′ and ITS4-R: 5′-TCC GCT TAT TGA TAT GC-3′). PCR amplification was performed using GeneAmp® PCR system 9700 (Applied Biosystem, Foster City, CA, USA) with a 20 µl reaction that comprised of 20 ng of genomic DNA template, 10× buffer with 25 mM $MgCl_2$, 10 mM DNTP's, 2U of Taq DNA polymerase and 10 pmol of each primer. PCR products were verified by 1% gel electrophoresis with a 100 bp DNA ladder. Sequence assembly was performed with Finch TV version 1.4.0 (www.geospiza.com). The obtained sequences were identified using a BLAST search against the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). Based on the initial ITS sequences blast results from the GenBank database, the endophyte CpE sequence and the endophyte sequences derived from NCBI (Supplementary Table 1) were used for phylogenetic analysis (Neighbor-joining method with 1000 bootstrap replication) using MEGA 6.

2.3. Effect of incubation temperature on CpE endophyte growth

An agar plug of actively growing CpE-mycelia inoculum was placed in the center of a perti dish containing PDA. CpE-inoculated PDA plates were incubated at different temperatures (30, 35, 40, 45, 50, 55, 60, 65 and 70 °C) for one week in a growth chamber. The fungal growth diameter was recorded.

2.4. Growth and CpE colonization assay

Cucumber seeds were surface-sterilized with 0.5% sodium hypochlorite for 3 min, then rinsed in sterile distilled water (SDW) for 10 min. Sterilized seeds were soaked in 50 ml conidial spore suspension (1×10^6) of *Thermomyces* isolate and SDW (Control) for 6 h. The primed seeds were sown in plastic pots containing mixture (2:1, v/v) of clay and sand infested with CpE conidial spores. Control pots received SDW. Seedlings were grown in the growth chamber at 25 ± 2 °C for three weeks before transferred into filed station under normal climatic conditions. Roots of emerged treated seedlings were examined for fungal endophytic colonization as described by Shivanna et al. (1996) at the same isolation conditions. Treated and control pots were divided into two groups, one group is subjected to water regime 9% field capacity while the other received 12% water field capacity.

2.5. Measurement of transpiration and photosynthesis parameters

Measurements of the maximum quantum efficiency of PSII photochemistry, photosynthesis rate, transpiration rate, leaf intercellular CO_2 concentration and stomatal conductance were performed by using infrared gas analyzer (IRGA, CI 350) photosynthesis system (CID Bio-Science, Inc.). Four individual plants were selected from each CpE treatment and untreated control. Measurements were done on the youngest fully expanded, healthy leaf. Measurement light level was set to PAR of 1500 µmol m^{-2} s^{-1}, which was provided by a LED light source. Relative humidity in the leaf chamber was 50%, and the air temperature was controlled at 26 ± 0.1 °C. The incoming air CO_2 level in the leaf chamber was set at 360 ppm. The leaf was positioned in the leaf chamber and allowed to acclimatize to the conditions in the

chamber. The analyses were taken after stomata and photosynthesis had stabilized, and the rates remained constant. WUE was determined using the following formula:

WUE = The current net CO_2 assimilation rate (*Pn*)/the current transpiration rate (*Tr*) (1)

2.6. Assays for PAL, SOD, CAT and POX activities

Frozen plant tissue (200 mg) was ground to a fine powder in a pre-cooled mortar and pestle, and 2 ml of extraction buffer [0.2 M phosphate buffer (pH 7.2), 0.1 mM EDTA, 1 mM DTT, and 2 U protease inhibitor cocktail] was added. The macerated suspension was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was collected and used as the source of enzyme.

PAL activity was assayed by measuring the *trans*-cinnamic acid formation at 290 nm using a UV-1800 UV–vis spectrophotometer (Genesys 5, Thermo Spectronic, Rochester, NY, USA), and calculated using a standard *trans*-cinnamic acid (Sigma-Aldrich) curve. The enzyme reaction mixture contained 100 mMTris- −HCl, 40 mM l-phenylalanine (Sigma-Aldrich), and an aliquot of the enzyme in a total volume of 1 ml. PAL activity was expressed in U g^{-1} FW according to Nagarathna et al. (1993).

SOD activity was determined by quantifying the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme according to Giannopolitis et al. (1977). The reaction mixture contained 50 mM sodium carbonate, 50 mM sodium phosphate buffer (pH 7.6), 0.1 mM EDTA, 50 µM NBT, 10 µM riboflavin, 12 mM L-methionine and 100 µl of crude extract in a final volume of 3.0 ml. The reaction mixture exposed to white light for 15 min at room temperature and the absorbance was recorded at 560 nm using a spectrophotometer A control reaction was performed without crude extract. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

POX activity was estimated using a UV/Vis spectrophotometer (Genesys 5, Thermo Spectronic, Rochester, NY, USA), as described previously (Kim and Yoo, 1996). The reaction mixture, which consisted of 0.8 ml of 0.2 M phosphate buffer (pH 7.2), 1 ml of 15 mM guaiacol, 1 ml of 3 mM hydrogen peroxide, and 0.2 ml of crude enzyme extract, was incubated at room temperature for 3 min. The absorbance of the colored product was monitored at 470 nm. POX activity expressed as Δ470 g^{-1} fresh weight (FW) min^{-1} was calculated using the following formula:

U/ml = [Change in absorbance min^{-1} × Reaction mixture volume (ml) × Dilution factor]/

[ε470 × Enzyme extract volume (ml)]

CAT activity was determined spectrophotometrically at 240 nm as described previously (Aebi, 1984). CAT activity expressed as Δ240 g^{-1} FW min^{-1} was calculated using the following formula, modified with hydrogen peroxide coefficient ε240:

U/ml = [Change in absorbance min^{-1} × Reaction mixture volume (ml) × Dilution factor]/

[ε240 × Enzyme extract volume (ml)]

2.7. Determination of total saponin

The dry materials were extracted according to the method described by Mostafa et al. (2013). Total saponin content was determined spectrophotometrically at 473 nm (Ebrahimzadeh and Niknam, 1998). Saponin contents were calculated based on the average value of absorbance at each concentration of the diosgenin standard.

2.8. Determination of total carbohydrate

The water-soluble carbohydrates and total carbohydrates were quantified by an anthrone-sulfuric acid method which carried out according to Fales, (1951) and Trevelyan et al. (1952). The developed blue-green color was read at the wavelength of 620 nm.

2.9. Determination of total flavonoids

The total flavonoid content of the plant extract was determined by the aluminum chloride colorimetric method using absorbance wavelength at 510 nm (Chang et al., 2002). The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight.

2.10. Determination of total proteins

Total soluble and insoluble proteins were determined according to the method adopted by Lowry et al. (1951).

2.11. Statistical analysis

Data obtained were subjected to a one-way analysis of variance (ANOVA). Significant differences between the control and treatments ($P \leq 0.05$) obtained by student *t*-test using SPSS software (SPSS20.0, SPSS Inc., USA). Values shown in the figures are the means ± standard errors (SEs) of four independent replicates. Correlation analysis was carried out using Corrplot in R project v.3.2.2.

3. Results

In our recent study, we were able to illustrate the significant roles of plant growth promoting fungi (PGPF) *Trichoderma longibrachiatum* isolated from desert soil in Egypt that confer beneficial agronomic traits to onion (*Allium cepa*) and induce disease resistance against *Fusarium oxysporum* cepa (Abdelrahman et al., 2016). This study extended the previous work to isolate prospective beneficial endophytic fungi suited for the arid climatic zone and their potential roles in mediating cucumber heat stress tolerance under field conditions.

3.1. Morphological, molecular examination and phylogenetic analyses

A thermophilic CpE fungus was isolated from the roots of an extreme hot desert-adapted delile plant, Aswan governorate, Egypt. The climatic environments in this region ranged between moderately cold dry winter to very hot dry summer (Abdelrahman et al., 2016). In addition, the soil composition in this desert area is well-graded sand with gravel and some calcareous remains (El-Shinawai and Naymushina, 2015). Pigment production, colony characteristics of the isolated fungus were recorded. Spores, mycelium characters, and fruiting structures were observed microscopically for morphological identification. The isolated CpE fungus displayed grey to black color hyphae with short unbranched-conidiophores. Conidia solitary was globose to subglobose or angular with dark brown color (Fig. 1). The isolate is identified as *Thermomyces lanuginosus* according to taxonomic keys of Moubasher, (1993). In addition, the growth-temperature curve of CpE isolate at different temperatures indicated the thermotolerance character of CpE endophyte (Fig. S1). The CpE isolate was able to grow up to 62 °C with maximum growth (12 cm) was recorded at 40–50 °C (Fig. S1).

The CpE isolate was studied in-depth for its molecular identification using ITS region. PCR amplification fragments of the ITS regions with the length between 587 and 595 bps were generated using ITS1 and ITS4 primers. These fragments were sequenced separately, and blast searched against NCBI Genbank database. The blast search results revealed high sequence similarity of CpE isolate (92% sequence identity

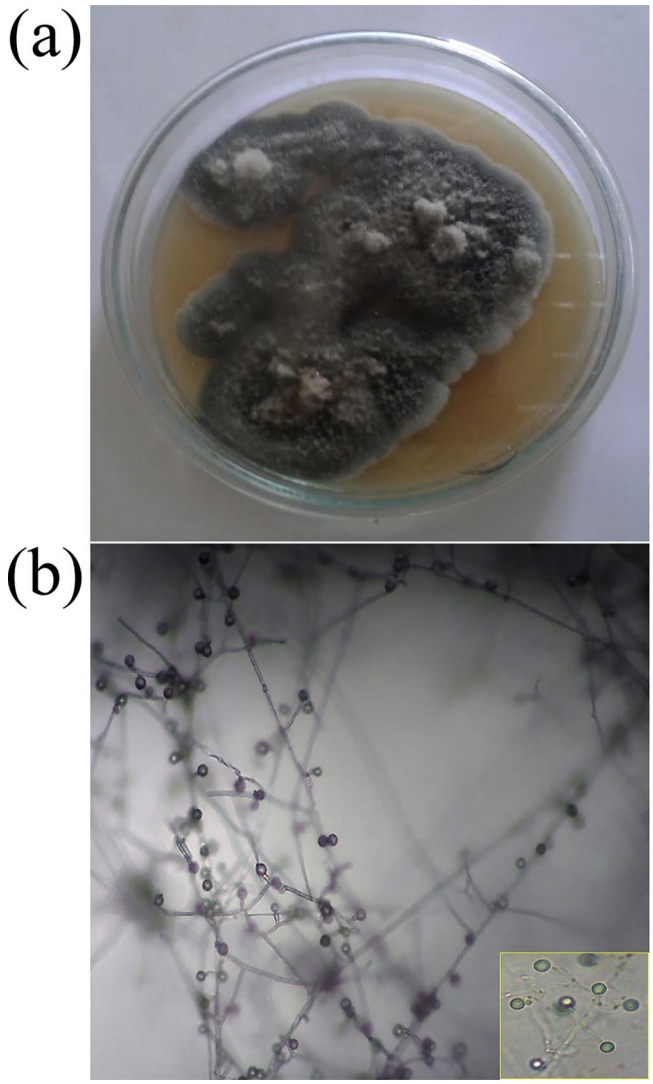

Fig. 1. Colony morphology of CpE isolate grows on potato dextrose agar media for seven days at 45 °C (**a**) and light microscope showing the CpE hyphae with short unbranched-conidiophores (**b**).

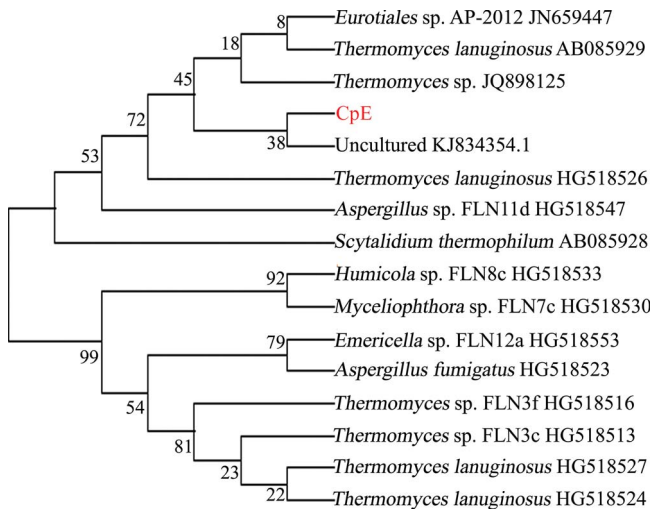

Fig. 2. Phylogenetic relationships of isolated CpE fungal endophyte and selected thermophilic fungi derived from NCBI Genbank based on nuclear ribosomal internal spacer sequence (ITS). The phylogram represents Neighbor-joining method with 1000 bootstrap replication. Bootstrap values are shown on each branch using MEGA 6 software.

and *E*-value 0.0) to *Thermomyces* sp. accession JQ898125.1 and uncultured fungus clone Z1C-9 accession KJ834354.1. Next, we use the nucleotide sequence alignments between CpE isolate and other thermophilic fungi derived from NCBI Genbank to construct a neighbor-joining phylogenetic tree using Mega 6 software (Fig. 2). The phylogenetic tree revealed a close genetic relationship between CpE isolate and other *Thermomyces* sp. that were isolated from the rhizosphere soils of different arid environments (Fig. 2).

3.2. Photosynthetic responses of cucumber plant-treated with CpE under heat stress

The photosynthetic responses, including P_n, *Fv/Fm*, T_r, C_i, G_s, and WUE were determined at 25 °C, 30 °C and 40 °C in the two-month-old cucumber plants-treated with CpE and untreated control plants (Fig. 3). There were no significant differences in the all photosynthetic responses between CpE-treated and untreated cucumber plants at 25 °C (Fig. 3). However, the increase in the ambient temperature from 25 °C to 40 °C resulted in significant changes in the photosynthetic process between CpE-treated and untreated cucumber plants (Fig. 3). The general trend in the P_n response to heat stress was similar between both CpE-treated and untreated cucumber plants with a significant decrease over time. Heat stress accelerated the P_n decline process in untreated cucumber plants, while the CpE treatment delayed the P_n decline process under respective conditions (Fig. 3). Similarly, heat stress resulted in a significant reduction pattern in maximum efficiency of PSII in both CpE-treated and untreated cucumber plants (Fig. 3). While CpE-treated cucumber plants exhibited a small reduction in PSII efficiency, the untreated cucumber plants displayed a sharp decrease. In contrast, heat stress significantly increased G_s and T_r in untreated cucumber plants. However, CpE-treated plants minimally changed, indicating that CpE-treated plant stomata responded slowly to heat stress relative to untreated cucumber plants (Fig. 3). In addition, WUE and leaf fresh weight as important indicators of plant fitness and vitality under heat stress were significantly higher in CpE-treated cucumber plants relative to untreated plants (Fig. 3). Whereas, there was no significant difference in Ci level in CpE-treated and untreated cucumber plants under heat stress conditions.

To understand the relationship between the different photosynthesis elements under heat stress conditions, Pearson correlation coefficient was carried out using Corrplot package in R (Fig. 4). A significant positive correlation was observed between G_s and T_r (r = 0.96), FW and WUE (r = 0.65) and P_n and *Fv/Fm* (r = 0.51). However, a negative correlation trend was detected between P_n and G_s (r = − 0.76), P_n and T_r (r = −0.74) and G_s and WUE (r = −0.63). This analysis indicated that heat stress has a profound effect on P_n rate through nonstomatal limitation in cucumber plants and the reduction in P_n was mostly associated with the disturbance in PSII reaction centers rather than stomatal movement.

3.3. Changes in cucumber metabolite contents and antioxidant activities in response to CpE treatment

We hypothesized that significant changes in the metabolite pool of CpE-treated cucumber plants might occur and these changes enhanced the cucumber plant survival capacity under heat stress condition. For this purpose, we analyzed the total saponin, flavonoid, carbohydrate and protein contents in CpE-treated and untreated cucumber plants (Fig. 5). The obtained results indicated that significant increases ($P < 0.05$) in the total saponin (3.07 mg g^{-1} DW), flavonoid (17.37 mg g^{-1} DW), soluble and insoluble carbohydrate (20.71 and 155.39 mg g^{-1} DW, respectively) and soluble protein (53.44 mg g^{-1} FW) contents were detected in CpE-treated cucumber plants relative to untreated control plants (Fig. 5). However, a significant ($P < 0.05$) decrease in total insoluble protein (33.90 mg g^{-1} FW) level was observed in CpE-treated cucumber plants relative to untreated control

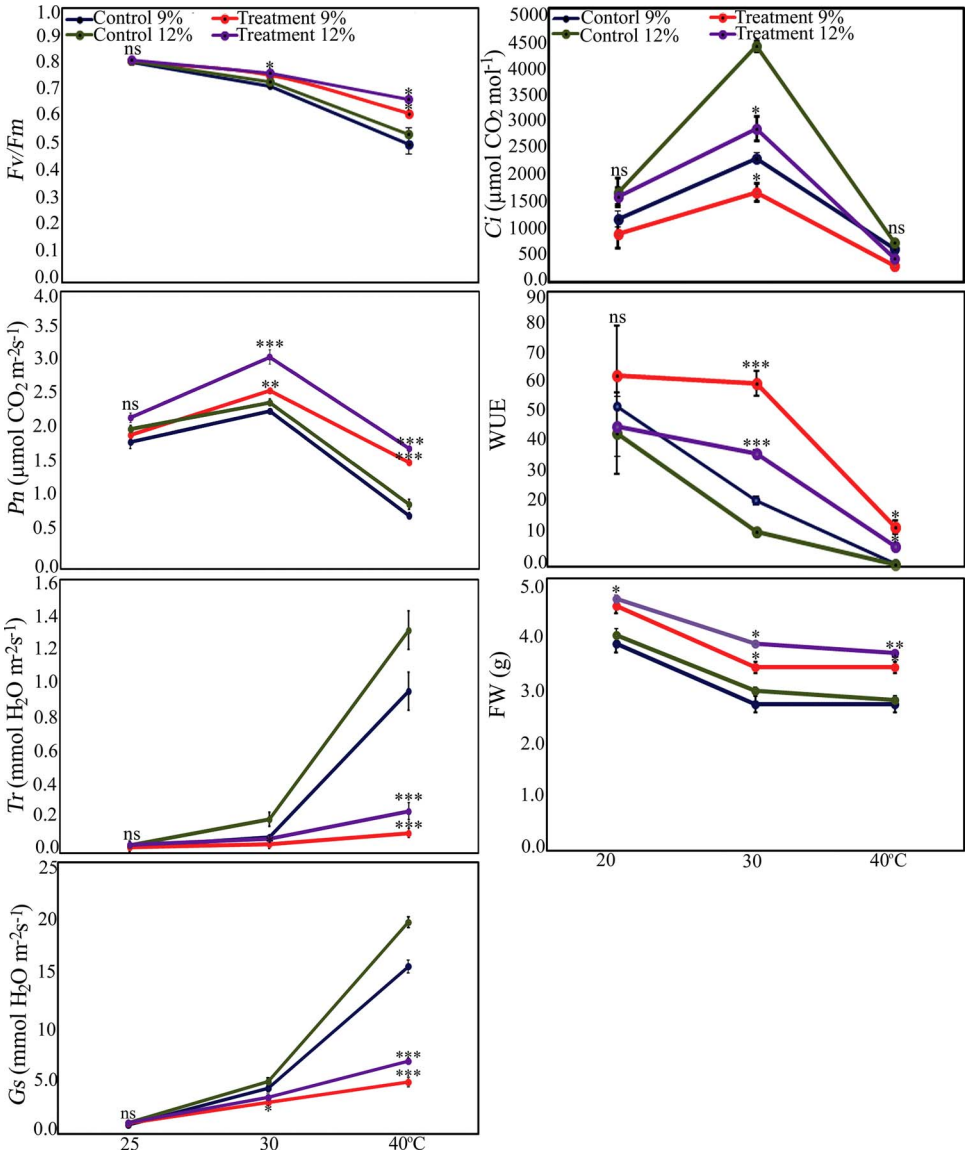

Fig. 3. Changes of photosynthetic parameters in cucumber plants-treated with fungal endophyte CpE and untreated control plants under heat stress conditions. Values are mean ± standard errors (SEs) of four independent biological replicates (n = 4). Maximum quantum efficiency of PSII photochemistry, *Fv/Fm*; photosynthetic rate, *Pn*; transpiration rate, *Tr*; leaf intercellular CO_2 concentration, *Ci*; stomatal conductance, *Gs* and water use efficiency, WUE. Asterisks indicate significant difference in CpE-treated and untreated cucumber plants, as determined by analysis of variance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

(Fig. 5).

Through evolution, plants have established a set of antioxidant systems to eliminate the excessive ROS level that generated by environmental cues (Li et al., 2016). To investigate the effect of CpE treatment on the cucumber antioxidant system, we analyzed the major antioxidant enzyme activities, including PAL, SOD, CAT and POX, in CpE-treated and untreated cucumber plants (Fig. 6). Significant increases in SOD, CAT and POX activities (66.66, 17.56 and 53.0 U g^{-1} FW, respectively) were detected in CpE-treated cucumber plants relative to untreated control (Fig. 6). However, no significant difference was observed in PAL activity between CpE-treated and untreated cucumber plants.

3.4. Cucumber root and leaf morphological changes in response to CpE treatment

The spatial arrangements of the root and its components, including length, number and position are essential aspects of plant plasticity and performance under abiotic stress conditions (Koevoets et al., 2016). In the present study, the CpE-treated cucumber plants exhibited a significant ($P < 0.02$) increase in the root length (6.73 cm) in comparison with untreated control plants (5 cm) (Fig. 7). In addition, the leaf FW/DW ratio was significantly ($P < 0.01$) higher in CpE-treated cucumber plants than the untreated control plants (10.82 and 6.66, respectively). These results were in accordance with the improvement in the leaf water content (2.39 mg g^{-1} FW) of CpE-treated cucumber plants relative to untreated control plants (1.47 mg g^{-1} FW).

4. Discussion

In the coming years, agriculture will have to deal with growing crops under suboptimal conditions accompanied by increased food demand, creating a gap between the yield potential and the current yield achievement (Koevoets et al., 2016). To meet these future challenges in crop production dictated by unpredicted climate changes, plant scientists and breeders need to develop new crop varieties and agricultural practices that are able to endure a wide range of abiotic stresses. Since the early 90s, the plant-endophytic fungi associations have been the subject of intensive research, allowing us to gain an insight into the critical role of fungal symbionts in plant physiology and ecology (Rozpądek et al., 2015). Recent studies indicate that the agronomic benefits conferred by endophytic fungi contribute to the plant adaptation to several abiotic stresses such as persistent drought, extreme temperatures, increased soil salinity and heavy metal toxicity, which has been proven to significantly affect plant growth and development (Singh et al., 2011; Khan et al., 2012; Hubbard et al., 2013;

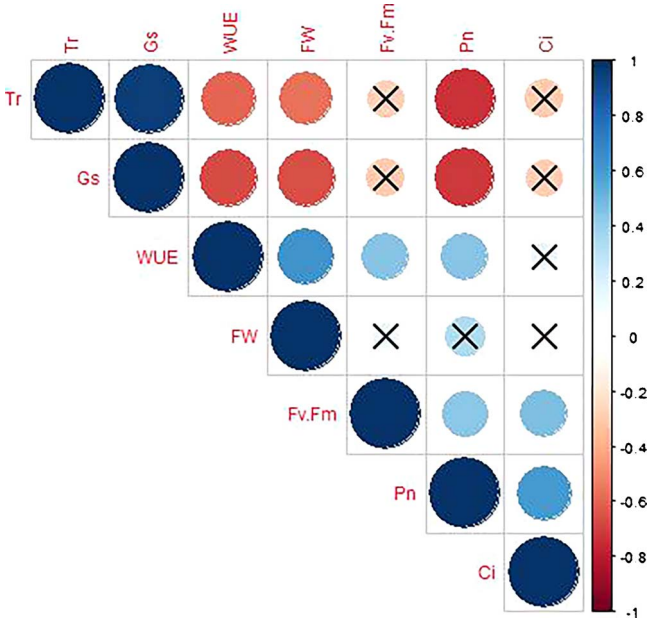

Fig. 4. Pearson correlation plot between photosynthesis parameters including maximum quantum efficiency of PSII photochemistry, *Fv/Fm*; photosynthetic rate, *Pn*; transpiration rate, *Tr*; leaf intercellular CO_2 concentration, *Ci*; stomatal conductance, *Gs* and water use efficiency, WUE. The color bar on the right side represents the significant R values.

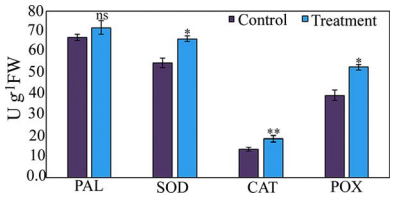

Fig. 6. Changes in antioxidant enzyme activity in CpE-treated and untreated cucumber plants. Phenylalanine ammonia lyase, PAL; sodium dismutase, SOD; catalase, CAT and peroxidase, POX. Values are means ± standard errors (SEs) of four independent replicates (n = 4). Asterisks indicate significant difference in CpE-treated and untreated cucumber plants, as determined by a Student's *t*-test (*P < 0.05; **P < 0.01; ***P < 0.001).

Yamaji et al., 2016). However, little is known about the host plant-endophytic fungi interactions in the arid desert ecosystems where water availability is insufficient. Given the potential for agricultural sustainability, there is a great interest in understanding the mechanisms of plant-endophytic interaction in the desert ecosystem. In the present study, a thermophilic endophyte CpE isolated from the roots of the hot desert-adapted delile plant was identified based on ITS sequence, revealing 92% sequence identity to *Thermomyces* sp. (Fig. 2). *Thermomyces* spp. are thermo-tolerant fungi that can survive under extreme temperatures up to 62 °C with high cellulases and xylanases enzymatic activities (Moretti et al., 2012). The mechanisms of thermal adaptation by *Thermomyces* were attributed to the presence of several DNA-related pathways, including ubiquitin degradation, histone acetylation/deacetylation, and poly ADP-ribosylation, which play important roles in cell signaling and DNA repair under heat stress condition (Mchunu et al., 2013). To date, there is no report about the beneficial role of this *Thermomyces* sp. in crop heat stress tolerance. Therefore, the physiological mechanisms of *Thermomyces*-mediated heat stress tolerance in cucumber plants grown under field conditions are an important aspect of agricultural research.

The adverse effects of heat stress on plant growth and crop yield were mainly caused by its negative impacts on the photosynthetic process, which is the most thermosensitive aspects of plant functions (Wang et al., 2016; Abdelrahman et al., 2017a). The results of our study showed a substantial decrease in photosynthesis process in response to heat stress on cucumber plants grown in the field (Fig. 3). Heat stress induced a sharp decline in maximum PSII efficiency and P_n process in control cucumber plants, while CpE-treated cucumber plants displayed an slow reduction, demonstrating that CpE treatment maintains PSII apparatus functional under heat stress in comparison with untreated control plants. PSII is believed to play a central role in the response of leaf photosynthesis to heat stress and any disorganization of PSII reaction centers will lead to a significant decrease in photosynthetic capability (Feng et al., 2014). Similarly, Hubbard et al. (2013)

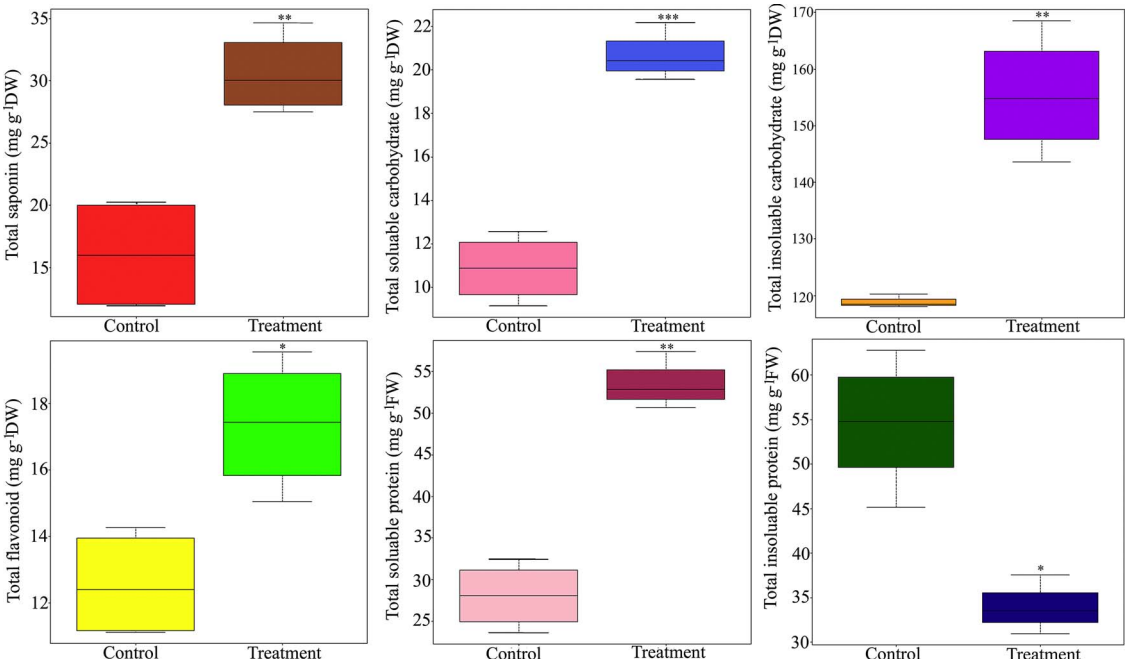

Fig. 5. Box plot diagram showing the changes in total saponin, flavonoid and carbohydrate contents (mg g^{-1} DW) and total protein contents (mg g^{-1} FW) in CpE-treated and untreated cucumber plants. Values represent the maximum, third quartile, median, first quartile and minimum of four independent replicates (n = 4). Asterisks indicate significant difference in CpE-treated and untreated cucumber plants, as determined by a Student's *t*-test (*P < 0.05; **P < 0.01; ***P < 0.001).

Fig. 7. The effect of CpE endophyte treatment on the cucumber root length in comparison with untreated control plant.

suggested that the application of endophytic fungi SMCD 2206 increased wheat (*Triticum aestivum*) heat stress tolerance by lowering photosynthetic stress and increasing maximum PSII efficiency in comparison with untreated control plants. Recently, Vahabi et al. (2016) suggested that endophytic fungus *Piriformospora indica* colonizes *Arabidopsis thaliana* roots promotes plant growth under high-light stress relative to untreated control plants, and *P. indica*-treated plants exhibited higher efficiency of the photosynthetic electron flow relative to untreated plants under light stress. It worth noting that PSII is severely damaged by high-temperature stress such as 45 °C rather than moderately temperature stress (Tang et al., 2007). Thus, we hypothesize that in addition to PSII damage, the decline in *Pn* rate in cucumber plants under heat stress conditions could also be attributed to the inhibition of Rubisco activase which is very sensitive to temperature changes, resulting in lower CO_2 assimilation. In the present study, CpE-treated cucumber plants showed a slow stomatal opening and low rate of transpiration under heat stress (Fig. 3). In contrast, heat stress induced a rapid increase in stomatal conductance and high rate of transpiration in untreated cucumber plants (Fig. 3). The obtained results indicate that CpE treatment maintains leaf water content, which was evident by improved WUE in the CpE-treated cucumber plants relative to untreated control (Fig. 3). These results indicate that CpE-treatment helps the cucumber plants in preventing hydraulic failure and excessive water loss through stomatal closure as a physiological-adaptive strategy to save water before further damages happen due to the increase in the temperature stress. In addition, WUE has a significant positive relationship with FW, which is an important physiological trait for yield improvement under limited water conditions (Polania et al., 2017). Our current findings confirm an earlier study by Khan et al. (2012) suggesting that the endophytic *Paecilomyces formosus* LHL10 induced salt stress tolerance in cucumber by increasing relative water contents and photosynthesis rate in comparison with untreated cucumber plants.

Endophytes proved to exert multiple physiological effects on the host plants, including photosynthesis, enzymatic activities and metabolites re-shaping (Yang et al., 2016). In the present study, CpE treatment promoted several primary and secondary metabolite accumulations, including total soluble and insoluble carbohydrate, saponin, flavonoid and soluble protein relative to untreated cucumber plants (Fig. 5). Similarly, inoculation of endophytic fungal strains, CXB-11 (*Nigrospora* sp.) and CXC-13 (*Fusarium* sp.) conferred better promotion effects in grape (*Vitis vinifera* L.) reducing sugar, total flavonoids and *trans*-resveratrol, demonstrating the opportunity of using endophytic fungi as fine-tuning regulator to outline grape quality (Yang et al., 2016). In addition, an endophytic association of *Penicillium minioluteum* and soybean (*Glycine max* L.) has significantly eliminated the adverse effects of salinity stress by inducing the biosynthesis of plant's hormones and flavonoids (Khan et al., 2011). In many plant-microbe interactions, the higher accumulations of sugars and flavonoids in plant tissues act as ROS-scavenger and signaling molecules, enabling plant growth and abiotic and biotic stress tolerance (Singh et al., 2011; Morkunas and Ratajczak, 2014; Abdelrahman et al., 2015, 2016). Therefore, we suggested that CpE treatment exerts multiple changes in cucumber metabolites and these alterations may confer the CpE-mediated heat stress tolerance in cucumber.

CpE treatment promoted the antioxidant systems in cucumber, which are well known physiological response for better protection against oxidative damage caused by various abiotic stresses (Abdelrahman et al., 2015). CpE-treated cucumber plants displayed a significant increase in SOD, CAT and POX activities relative to untreated cucumber plants (Fig. 6). A similar finding was reported by Sun et al. (2010) suggesting that *Piriformospora indica* confers drought tolerance in Chinese cabbage (*Brassica rapa* Subsp *chinensis*) leave by stimulating POX, CAT and SOD antioxidant enzymes (Sun et al., 2010). Improved antioxidant enzyme activities were also reported in the beneficial effects of mycorrhizal colonization on plants grown under arid and semi-arid environments (Ruiz-Lozano et al., 2001; Singh et al., 2011). CpE treatment induced antioxidant enzyme activities in cucumber plants, which can enhance membrane stability against oxidative stress generated by ROS accumulation under heat stress conditions. In addition, CpE-treated cucumber plant exhibited an improvement in root length relative to untreated cucumber plants (Fig. 7). This phenological response is essential adaptive trait in the desert ecosystems, enabling the plant for better penetration and extraction of soil moisture and nutrients under limited water conditions. In support of our finding, a recent study evaluated the effects of the root-colonizing endophytic fungus *P. indica* on wheat under drought and mechanical stress showed that the colonized plants had greater root length and relative water contents under stressful conditions due to higher absorption sites for water and nutrients in comparison with non-colonized plants (Hosseini et al., 2017).

5. Conclusion

A thermophilic endophytic fungus CpE was isolated and identified using molecular characterization, revealing a close genetic relationship to *Thermomyces* sp. isolated from different arid land environments. The beneficial effects of CpE-mediated heat stress tolerance in cucumber plants were evaluated using photosynthetic responses, antioxidant enzyme activities and changes in the metabolite level in CpE-treated and untreated cucumber plants. In addition, an improvement in CpE-treated cucumber root length was observed. In general CpE-treated cucumber plant displayed a better adaptation to heat stress which will facilities the cultivation of a heat stress-tolerant cucumber in arid land environments.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

All the authors declare there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apsoil.2017.11.004.

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